On a new proposed mechanism of 5-fluorouracil-mediated cytotoxicity

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Abstract

The major molecular mode of action of the cytotoxic drug 5FU is generally considered to result from thymidylate synthase inhibition. Recent findings relating to the function of the human uracil-5 methyltransferase, TRMT2A, and its interaction with 5FU metabolites incorporated within tRNAs, lead to an additional hypothesis which is proposed here.

The synthetic uracil analogue 5-fluorouracil (5FU) is an important chemotherapeutic drug with a cytotoxic effect associated with an increased susceptibility to DNA breaks [1, 2]. The compound is converted to several active metabolites within cells including the nucleotides fluoro-deoxyuridine-monophosphate (FdUMP), fluoro-deoxyuridine-triphosphate (FdUTP), and fluoro-uridine-triphosphate (FUTP). The mechanism of action of 5FU and its cellular derivatives have been widely studied, and an inhibition of the deoxyuridine-monophosphate (dUMP) to deoxythymidine-monophosphate (dTMP or 'thymidylate') conversion enzyme, thymidylate synthase, is generally considered to be the major route toward cytotoxicity [1]. FdUMP binds irreversibly to the nucleotide-binding pocket of thymidylate synthase thus inhibiting dUMP to dTMP conversion. Although dTMP can be readily salvaged from thymidine via action of the thymidine kinase enzyme, thymidylate synthase inhibition-induced nucleotide imbalances can still lead to some misincorporation of deoxyuridine-triphosphate (dUTP) and FdUTP into DNA. The rectification of such misincorporation can usually be mediated by DNA repair excision enzymes for example, however, futile rounds of misincorporation-excision repair may ensue which might potentially lead to the formation of DNA breaks and thus cell death [1]. The actual details of thymidylate synthase inhibition-induced nucleotide misincorporation in the induction of DNA breaks and their inefficient repair however remain poorly characterised [1, 2]. Parallel observations that 5FU treatment leads to impaired repair of DNA breaks by homologous recombination [3], are nonetheless worth noting [2].

Also to be considered, is the fact that the FUTP nucleotide is extensively incorporated into RNA in direct competition with the natural uridine-triphosphate (UTP) nucleotide during gene transcription. The idea that this may contribute to the cytotoxic effect of 5FU is supported by the observation of a strong correlation between FUTP incorporation into RNA and attenuation of cologenic survival in multiple cancer cell lines [4, 5]. Although misregulated pre-rRNA processing and pre-mRNA splicing events for example have since been associated with the presence of FUTP in RNA, whether any of these play significant roles in 5FU-induced cytotoxicity remains to be elucidated [1].

A recent study by Carter et al. has demonstrated that the human uracil-5 methyltransferase (U5MT), TRMT2A, targets uridine-54 of tRNAs as a substrate for methylation [6]. The investigations involved revealing that cellular 5FU exposure led to extensive and irreversible

crosslinking between the TRMT2A enzyme and target ribonucleotides within tRNAs. The biochemical mechanism of the relevant methylation reaction was indeed previously known to occur via an enzyme-substrate covalently crosslinked complex [7]. Normally, such a complex is only a transient intermediate in the catalytic reaction and is accordingly quickly resolved thus releasing the methylated RNA and regenerating the methyltransferase [7] (illustrated schematically in Figure 1A). However, the incorporation of FUTP into U5MT target RNA sites leads to the enzyme becoming permanently crosslinked to the analogue ribonucleotide during the methylation reaction [6, 7] (Figure 1B). This principle was used to map the precise enzymatic target sites of TRMT2A in cellular RNAs by Carter et al., but the observations also raise the question of what biological effect the relevant unnatural irreversible crosslinking might have within the cell.

Although TRMT2A was found to target numerous tRNA isoacceptors [6], as the cellular pool of each isoacceptor is significantly larger than the number of cellular TRMT2A molecules, the deleterious effect of 5FU-mediated TRMT2A-tRNA crosslinking on overall tRNA function is probably quite limited. Nonetheless, what about the potential effects of TRMT2A function inhibition? This is a particularly relevant question to ask as we know of prominent parallel examples where cellular exposure to the cytidine analogue, 5-azacytidine, leads to permanent crosslinking of DNA cytosine-5 methyltransferases to DNA (in fact via a similar enzymatic crosslinking mechanism as observed for TRMT2A-RNA), thus leading to functionally relevant depletion/inhibition of the DNA methylation enzymes. Could an analogous 5FU-mediated TRMT2A inhibition lead to a significant cytotoxic effect? If we're considering just cellular loss of the methyltransferase functions of the enzyme, then probably not, as eukaryotic cells lacking tRNA uridine-54 methylation exhibit only very mild observable phenotypes [8]. It is intriguing however that while inactivation of the methyltransferase activity of the bacterial homolog of TRMT2A, TrmA, only leads to mild effects too, full disruption of the TrmA gene is lethal [9].

Indeed, while the apparently non-essential uridine-54 methylation in tRNAs might help improve rates of tRNA structural folding [10], circumstantial evidence indicates that the yeast homologue of TRMT2A, an alias of which includes NUD1, may have crucial orthogonal cellular roles, namely in homologous recombination-mediated DNA break repair [11-13]. Notably, such studies demonstrated NUD1 to harbour a DNA endo-exonuclease enzymatic

activity which might potentially participate in an early step of the homology-directed repair process [11, 12]. Although ectopic expression of NUD1 in mammalian cells was found to promote extrachromosomal homologous recombination processes [12], a role for TRMT2A itself in homology-directed DNA break repair however remains under-investigated and currently only speculative. Nonetheless, the recent independent characterisations that TRMT2A is a predominantly nuclear protein, and has a cell-cycle regulated expression profile which peaks in S-phase [14, 15], should help further support studies into its roles in DNA break repair. Certainly, there is no obvious reason as to why the expression of a tRNA methyltransferase, whose respective activity is to promote tRNA maturation, should peak in S-phase, although an additional role in homology-directed DNA repair for example would offer adequate explanation. A relevant important role in homologous recombination repair would also be further attractive overall, as it could explain lethality associated with TRMT2A homolog deletion being observed currently only in bacteria [9], where alternative compensatory routes of DNA break repair are much more limited compared to that present in eukaryotic cells.

When considering potential links between TRMT2A and 5FU-mediated cytotoxicity, both cellular and biochemical assays assessing the direct roles of TRMT2A in homology-directed DNA break repair, and the effects of its disruption/inhibition in sensitising cells to DNA breaks, should be a focus of further investigation. As the yeast studies on NUD1 function have suggested that it might play an early role in the homologous recombination repair process, this may guide efforts in elucidating any potential relevant roles for TRMT2A. Should the involvement of TRMT2A in homology-directed DNA break repair be positively established, then TRMT2A inhibition could well emerge as a major mechanism of 5FU-mediated cytotoxicity (Figure 2).

Figure legends

Figure 1. Schematic representations of the tRNA uracil-5 methylation reaction. (a) A target uridine nucleotide within tRNA is the normal substrate of a U5MT enzyme (such as TRMT2A for example) (step 1). During the catalytic reaction, the U5MT becomes covalently crosslinked to the carbon at position 6 of the uracil ring (step 2). This results in the carbon at

position 5 becoming susceptible to electrophilic attack, and a methyl group can be conjugated to it (step 3). U5MT-dependent proton abstraction from the 5-carbon occurs next (step 4), before release of the U5MT from the 6-carbon can occur (step 5). (b) When a fluoro-uridine nucleotide is present at the target position of the tRNA, U5MT-tRNA covalent crosslinking (step 2) and methyl group conjugation (step 3) both proceed normally, but the U5MT cannot mediate fluoride abstraction from the 5-carbon, and thus U5MT release from the 6-carbon is also blocked.

Figure 2. Hypothesised TRMT2A-inhibition route of 5FU-mediated cytotoxicity. The depicted events take place in the cell nucleus, where tRNA uridine-54 methylation, an early step in tRNA maturation, is known to occur. (a) Under normal conditions, the enzymatic interaction of TRMT2A with tRNAs is only very transient, thus maintaining a functional pool of TRMT2A in the nucleus able to participate in the repair of DNA breaks. (b) Under 5FU conditions, permanent crosslinks form extensively between TRMT2A and tRNAs molecules, thus almost certainly rendering the complexes non-functional. As cellular tRNA pools are large compared to the TRMT2A pool, the remaining un-crosslinked tRNAs are likely sufficient to maintain normal cellular protein synthesis. However, the severely depleted functional TRMT2A pool is not able to maintain normal DNA break repair, which can lead to cell death. The overall proposal will likely hinge on establishing the extent to which TRMT2A plays direct roles in the repair of DNA breaks.

References

1. Longley, D.B. et al. (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3 (5), 330-8.
2. Wyatt, M.D. and Wilson, D.M., 3rd (2009) Participation of DNA repair in the response to 5-fluorouracil. Cell Mol Life Sci 66 (5), 788-99.
3. Srinivas, U.S. et al. (2015) 5-Fluorouracil sensitizes colorectal tumor cells towards double stranded DNA breaks by interfering with homologous recombination repair. Oncotarget 6 (14), 12574-86.
4. Kufe, D.W. and Major, P.P. (1981) 5-Fluorouracil incorporation into human breast carcinoma RNA correlates with cytotoxicity. J Biol Chem 256 (19), 9802-5.
5. Glazer, R.I. and Lloyd, L.S. (1982) Association of cell lethality with incorporation of 5-fluorouracil and 5-fluorouridine into nuclear RNA in human colon carcinoma cells in culture. Mol Pharmacol 21 (2), 468-73.
6. Carter, J.M. et al. (2019) FICC-Seq: a method for enzyme-specified profiling of methyl-5-uridine in cellular RNA. Nucleic Acids Res.
7. Kealey, J.T. et al. (1994) Enzymatic mechanism of tRNA (m5U54)methyltransferase. Biochimie 76 (12), 1133-42.

8. Hopper, A.K. et al. (1982) Defects in modification of cytoplasmic and mitochondrial transfer RNAs are caused by single nuclear mutations. Cell 28 (3), 543-50.
9. Persson, B.C. et al. (1992) The gene for a tRNA modifying enzyme, m5U54-methyltransferase, is essential for viability in Escherichia coli. Proc Natl Acad Sci U S A 89 (9), 3995-8.
10. Davanloo, P. et al. (1979) Role of ribothymidine in the thermal stability of transfer RNA as monitored by proton magnetic resonance. Nucleic Acids Res 6 (4), 1571-81.
11. Asefa, B. et al. (1998) Genetic analysis of the yeast NUD1 endo-exonuclease: a role in the repair of DNA double-strand breaks. Curr Genet 34 (5), 360-7.
12. Semionov, A. et al. (1999) Transient expression of Saccharomyces cerevisiae endo-exonuclease NUD1 gene increases the frequency of extrachromosomal homologous recombination in mouse Ltk- fibroblasts. Mutat Res 435 (2), 129-39.
13. Choudhury, S.A. et al. (2007) Functional and genetic analysis of the Saccharomyces cerevisiae RNC1/TRM2: evidences for its involvement in DNA double-strand break repair. Mol Cell Biochem 300 (1-2), 215-26.
14. Guarguaglini, G. et al. (1997) Expression of the murine RanBP1 and Htf9-c genes is regulated from a shared bidirectional promoter during cell cycle progression. Biochem J 325 (Pt 1), 277-86.
15. Chang, Y.H. et al. (2019) TRMT2A is a novel cell cycle regulator that suppresses cell proliferation. Biochem Biophys Res Commun 508 (2), 410-415.